

=> d his

(FILE 'HOME' ENTERED AT 11:00:23 ON 30 MAR 2007)  
FILE 'CA' ENTERED AT 11:00:33 ON 30 MAR 2007  
L1 2472 S (IMMUNO? OR ANTIGEN OR ANTIBODY)AND(FRET OR ENERGY(2A)TRANSFER? OR  
(DONOR AND ACCEPTOR))  
L2 1453 S L1 AND (FLUOROPHOR? OR FLUORESC?)  
L3 25 S (RATIOMET? OR RATIO(4A)(INTENSITY OR EMIT OR EMITTED OR EMISSION  
OR FLUORESCENC?)) AND L1  
L4 96 S ((DUAL OR 2 OR TWO OR DOUBLE OR PAIR)(4A)(LABEL OR DYE OR  
INDICATOR OR FLUOROPHOR? OR FLUORESC?(1A)(TAG OR MOLECULE OR  
MOIETY))) AND L2  
L5 10 S L4 AND HOMOGEN?  
L6 1 S L4 AND (NONCOMPET? OR NON COMPET?)  
L7 33 S L3,L5-6  
L8 22 S L7 AND PY<2003  
FILE 'BIOSIS' ENTERED AT 11:11:50 ON 30 MAR 2007  
L9 10 S L8  
FILE 'MEDLINE' ENTERED AT 11:12:16 ON 30 MAR 2007  
L10 12 S L8  
FILE 'CA, BIOSIS, MEDLINE' ENTERED AT 11:12:52 ON 30 MAR 2007  
L11 24 DUP REM L8 L9 L10 (20 DUPLICATES REMOVED)

=> d bib,ab l11 1-24

L11 ANSWER 7 OF 24 CA COPYRIGHT 2007 ACS on STN  
AN 135:13785 CA  
TI Development of a ubiquitin transfer assay for high throughput screening  
by **fluorescence resonance energy transfer**  
AU Boisclair, Michael D.; McClure, Christopher; Josiah, Serene; Glass,  
Susan; Bottomley, Steve; Kamerkar, Shubi; Hemmila, Ilkka  
CS GPC Biotech, Inc., Cambridge, MA, USA  
SO Journal of Biomolecular Screening (2000), 5(5), 319-328  
AB An assay based on **fluorescence resonance energy transfer (FRET)** has been  
developed to screen for ubiquitination inhibitors. The assay measures  
the transfer of ubiquitin from Ubc4 to HECT protein Rsc 1083. Secondary  
reagents (streptavidin and **antibody** to glutathione-S-transferase [GST]),  
pre-labeled with **fluorophores** (europium chelate, Eu3+, and  
allophycocyanin [APC]), are noncovalently attached via tags (biotin and  
GST) to the reactants (ubiquitin and Rsc). When Rsc is ubiquitinated,  
Eu3+ and APC are brought into close proximity, permitting **energy**  
**transfer** between the **two fluorescent labels**. **FRET** was measured as time-  
resolved **fluorescence** at the emission wave-length of APC, almost  
entirely free of nonspecific **fluorescence** from Eu3+ and APC. The **FRET**  
assay generated a lower ratio of signal to background (8 vs. 31) than an  
assay for the same ubiquitination step that was developed as a dissoçn.-  
enhanced lanthanide fluoroimmunoassay (DELFI A). However, compared to  
the DELFI A method, use of **FRET** resulted in higher precision (4% vs. 11%  
intraplate coeff. of variation). Quenching of **fluorescence** was minimal  
when compds. were screened at 10 µg/mL using **FRET**. Employing a quick  
and simple **homogeneous** method, the **FRET** assay for ubiquitin transfer is  
ideally suited for high throughput screening.

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STN INTERNATIONAL LOGOFF AT 11:14:06 ON 30 MAR 2007

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(FILE 'HOME' ENTERED AT 07:19:12 ON 30 MAR 2007)

FILE 'CA' ENTERED AT 07:19:20 ON 30 MAR 2007

L1 41231 S HOLOCARBONIC OR APOCARBONIC OR HOLOENZYME OR APOENZYME OR  
HOLOPROTEIN OR APOPROTEIN OR APO OR HOLO  
L2 29429 S RATIOMET? OR RATIO(4A) (INTENSITY OR EMIT OR EMITTED OR EMISSION  
OR FLUORESCENC?)  
L3 86722 S (ZINC OR ZN OR ZN2) (5A) (DETECT? OR DETERMIN? OR ASSAY? OR ANALY?  
OR ASSESS? OR TEST? OR MEASUR? OR MONITOR? OR ESTIMAT? OR EVALUAT?  
OR SENSE# OR SENSOR OR SENSING OR PROBE# OR PROBING OR JUDG? OR  
QUANTITAT? OR QUANTIF? OR ASCERTAIN?)  
L4 56 S L1 AND L2  
L5 314 S L2 AND ENZYM?  
L6 392 S L2 AND L3  
L7 6611 S L2 AND FLUORESC?  
L8 208 S L7 AND (2 OR DUAL OR TWO OR MULTIPLE OR PLURAL?) (4A) (WAVELENGTH  
OR FREQUENCY)  
L9 33867 S SULFONAMI?  
L10 95 S L3 AND L9  
L11 50585 S (DUAL OR 2 OR TWO OR DOUBLE OR PAIR) (4A) (LABEL OR DYE OR  
INDICATOR OR FLUOROPHOR? OR FLUORESC? (1A) (TAG OR MOLECULE OR  
MOIETY))  
L12 19 S L2 AND L9  
L13 2 S L8 AND L9  
L14 71 S L1 AND L11  
L15 81886 S (DUAL OR 2 OR TWO OR DOUBLE OR PAIR) (4A) (LABEL? OR DYE OR  
INDICATOR OR FLUOROPHOR? OR FLUORESC? (1A) (TAG OR MOLECULE OR MOIETY  
OR GROUP))  
L16 466 S L2 AND L15  
L17 16 S L5 AND L16  
L18 35 S L8 AND L16  
L19 0 S L9 AND L16  
L20 257 S L1 AND L15  
L21 336 S L3 AND L15  
L22 0 S L5 AND L21  
L23 31 S L5 AND (FRET OR ENERGY(2A) TRANSFER? OR DONOR OR ACCEPTOR)  
L24 20 S L6 AND (FRET OR ENERGY(2A) TRANSFER? OR DONOR OR ACCEPTOR)  
L25 624 S L7 AND (FRET OR ENERGY(2A) TRANSFER? OR DONOR OR ACCEPTOR)  
L26 16 S L8 AND L25  
L27 17 S L20 AND (FRET OR ENERGY(2A) TRANSFER? OR DONOR OR ACCEPTOR)  
L28 8 S L21 AND (FRET OR ENERGY(2A) TRANSFER? OR DONOR OR ACCEPTOR)  
L29 343 S L4, L10, L12-14, L17-19, L22-24, L26-28  
L30 252 S L29 AND PY<2003  
FILE 'BIOSIS' ENTERED AT 08:14:58 ON 30 MAR 2007  
L31 169 S L30  
FILE 'MEDLINE' ENTERED AT 08:16:35 ON 30 MAR 2007  
L32 166 S L30  
FILE 'CA, BIOSIS, MEDLINE' ENTERED AT 08:18:06 ON 30 MAR 2007  
L33 376 DUP REM L30 L31 L32 (211 DUPLICATES REMOVED)

=> d bib,ab 133 1-376

L33 ANSWER 223 OF 376 CA COPYRIGHT 2007 ACS on STN  
AN 116:210724 CA  
TI Detection of analytes using fluorescent **energy transfer**  
IN Tsien, Roger Y.; Taylor, Susan S.; Adams, Stephen R.; Ji, Ying  
PA University of California, Oakland, USA  
SO PCT Int. Appl., 38 pp.  
PI WO 9200388 A1 19920109 WO 1991-US4676 19910701  
US 5439797 A 19950808 US 1993-114103 19930830  
PRAI US 1990-547990 A 19900702  
AB Analytes such as cAMP, GTP, hormone-receptor complexes, Ca<sup>2+</sup>, diacylglycerol, and phorbol esters are detd. by a method involving radiationless **energy transfer** between 2 fluorochromes, each bound to a protein; the proteins are reversibly assocd. with one another, the equil. between assocd. and dissocd. states being dependent on the analyte concn. Thus, the catalytic subunit of cAMP-dependent protein kinase (I) was labeled with FITC and the regulatory subunit of I with tetramethylrhodamine isothiocyanate, and the 2 subunits were allowed to assoc. to form **holoenzyme**. CAMP was detd. in single smooth muscle cells by microinjection of the cells with doubly labeled **holo-I**, illumination of the cells at 490 nm, and measurement of the **ratio of emitted light intensity** at 500-530 nm (fluorescein emission) and 580 nm (tetramethylrhodamine emission). The ratio rapidly increased after microinjection of isoproterenol ( $\beta$ 2-adrenergic agonist which raises cAMP concn.) or forskolin (adenylate cyclase activator) and decreased by propranolol ( $\beta$ 2-adrenergic antagonist). Expression of the genes for the catalytic and regulatory subunits of I in Escherichia coli, purifn. of the recombinant subunits, and purifn. of the catalytic subunit of I from porcine heart for use in the cAMP assay are described.

L33 ANSWER 237 OF 376 MEDLINE on STN  
AN 92035562 MEDLINE  
TI Multianalyte microspot immunoassay--microanalytical "compact disk" of the future.  
AU Ekins R P; Chu F W  
CS Department of Molecular Endocrinology, University College and Middlesex School of Medicine, London, U.K.  
SO Clinical chemistry, (1991 Nov) Vol. 37, No. 11, pp. 1955-67.  
AB Throughout the 1970s, controversy centered both on immunoassay "sensitivity" per se and on the relative sensitivities of labeled antibody (Ab) and labeled analyte methods. Our theoretical studies revealed that RIA sensitivities could be surpassed only by the use of very high-specificity nonisotopic labels in "noncompetitive" designs, preferably with monoclonal antibodies. The time-resolved fluorescence methodology known as DELFIA--developed in collaboration with LKB/Wallac--represented the first commercial "ultrasensitive" nonisotopic technique based on these theoretical insights, the same concepts being subsequently adopted in comparable methodologies relying on the use of chemiluminescent and **enzyme** labels. However, high-specific-activity labels also permit the development of "multianalyte" immunoassay systems combining ultrasensitivity with the simultaneous measurement of tens, hundreds, or thousands of analytes in a small

biological sample. This possibility relies on simple, albeit hitherto-unexploited, physicochemical concepts. The first is that all immunoassays rely on the measurement of Ab occupancy by analyte. The second is that, provided the Ab concentration used is "vanishingly small," fractional Ab occupancy is independent of both Ab concentration and sample volume. This leads to the notion of "ratiometric" immunoassay, involving measurement of the ratio of signals (e.g., fluorescent signals) emitted by **two labeled** Abs, the first (a "sensor" Ab) deposited as a microspot on a solid support, the second (a "developing" Ab) directed against either occupied or unoccupied binding sites of the sensor Ab. Our preliminary studies of this approach have relied on a dual-channel scanning-laser confocal microscope, permitting microspots of area 100 microns<sup>2</sup> or less to be analyzed, and implying that an array of 10(6) Ab-containing microspots, each directed against a different analyte, could, in principle, be accommodated on an area of 1 cm<sup>2</sup>. Although measurement of such analyte numbers is unlikely ever to be required, the ability to analyze biological fluids for a wide spectrum of analytes is likely to transform immunodiagnostics in the next decade.

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STN INTERNATIONAL LOGOFF AT 08:19:55 ON 30 MAR 2007